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Synthesis and Properties of Di-*n*-dodecyl α,ω-Alkyl Bisphosphate Surfactants

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Three gemini and two bolaform bisphosphate surfactants of the type 12-*s*-12, with $s = 6$, 8, 12, 18, and 24 carbon atoms, have been synthesized and their aggregation behavior has been studied. The bolaform surfactants 12-18-12 and 12-24-12 were found to form vesicles in aqueous solution, as indicated by electron microscopy. The geminis 12-6-12, 12-8-12, and 12-12-12 form micellar structures. The cmc's of the geminis, obtained from conductivity measurements, spectroscopic methods, and microcalorimetry, are very low, on the order of 10^{-4} to 10^{-5} M. The cmc decreases with increasing spacer length. For the bolaform amphiphiles 12-18-12 and 12-24-12 noncooperative phase transitions are detected using fluorescence depolarization and DSC. NMR line-broadening studies display unusual behavior. The spacer within the 12-24-12 vesicles has been found to be membrane spanning, as confirmed by X-ray powder diffraction.

Introduction

Bolaform-type and gemini-type surfactants have gained much interest over the last years, because of their unusual aggregation properties,[1−10] compared to those of the corresponding conventional surfactants. Bolaform amphiphiles are found in natural membranes of archaebacteria, a class of organisms that live in habitats with extreme circumstances, such as high temperatures, low pH values, and saturated salt environments.[11] When there is a single spacer, this spacer is longer than the other chains and is therefore membrane spanning in such membranes. Other types of bolaform membranes consist of two membrane-spanning spacers. As a result, these membranes possess an unusual thermodynamic stability. They display a very slow lateral and transversal diffusion, and they resist fusion. Several synthetic bolaform amphiphiles have been studied.[10,12−21] They were found to form monolayer structures with a membrane-spanning spacer as well. In a few cases, backfolding of the spacer has been observed.[3,22−24]

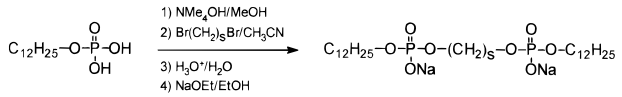

Figure 1. Synthetic sequence for the preparation of the 12-*s*-12 surfactants. $s = 6$, 8, 12, 18, and 24.

Gemini surfactants have much shorter spacer lengths. They form micellar structures in aqueous solution due to considerable backfolding of the spacer.[3,6,10] Their properties are different from those of single-chained micelle-forming amphiphiles. The most pronounced differences are the extremely low cmc's and low Krafft points.[4] Zana *et al.*[5−10] have reported studies on *m*-*s*-*m* type geminis with two tetraalkylammonium headgroups. They found that geminis with short spacers (C_2 and C_3 chains) form threadlike micelles and that those with spacers of medium length (C_5−C_{12}) form spherical micelles.[10] Geminis with rigid spacers, which cannot fold back, display other unusual properties.[3] For these geminis, premicellar aggregates are believed to be formed at concentrations just below the cmc. Despite rather extensive studies, their exact aggregation behavior in aqueous solution is still not fully understood.

We became highly interested in these new types of amphiphiles, because of several potential applications, such as, for instance, drug targeting. For this reason, we wanted to synthesize bolaform- and gemini-type surfactants which would have a membrane spanning spacer within a vesicular aggregate. In this paper, we report the synthesis and some physical properties of three gemini-type and two bolaform-type amphiphiles with two phosphate headgroups, two dodecyl chains, and one connecting spacer of variable length *s* (Figure 1).

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Following the nomenclature used by Zana *et al.*,[6] the amphiphiles will be called *m*-*s*-*m* with $m = 12$. As no clear distinction has been made so far in the literature between bolaform and gemini surfactants, we propose to define bolaform surfactants as those having a spacer longer than the other chains ($s > 12$ in the present work) and gemini surfactants as those with shorter spacers. This is a useful definition in light of our results (see Results and Discussion), which prove that clear relationships between molecular and aggregate structure exist.

Results and Discussion

Synthesis. The novel amphiphiles were synthesized according to the method described by Bauman,[25] as outlined in Figure 1. In all cases, the chemical yields were excellent. The surfactants were all converted into their disodium salts and checked using IR spectroscopy[26] prior to the experiments.

Electron Microscopy. For the geminis 12-6-12, 12-8-12, and 12-12-12, no vesicular structures were found with either transmission electron microscopy (TEM) or freeze fracture electron microscopy (FFEM). Similar results were obtained by Zana *et al.*[6] for the corresponding dimeric ammonium amphiphiles.

For the bolaform amphiphiles 12-18-12 and 12-24-12 unilamellar and multilamellar vesicles were observed with both TEM and FFEM. Some micrographs are shown in Figure 2. The vesicles varied in size between 150 and 700 nm. Vesicles prepared with the ethanol injection method[27] (Experimental Section) were smaller than those prepared by simply stirring the surfactant solution. This is a common phenomenon.[28] The vesicular solutions were stable above their T_c for several months, as monitored by TEM and by visual inspection of the slightly turbid solutions. No differences could be detected between the samples prepared in double-distilled water and those prepared in aqueous NaAc/HEPES buffers (pH = 7.4).

Phase Behavior. The phase transition temperatures (T_c) were determined by fluorescence depolarization,[29] DSC,[13,30−32] and NMR.[14,27,33] The results are summarized in Table 1.

The method of vesicle preparation (Experimental Section) had no effect on the results. With fluorescence depolarization, a gradual, noncooperative transition was found for 12-18-12 and 12-24-12, as is shown in Figure 3. The transition range was 22−75 °C for 12-18-12 and 26−64 °C for 12-24-12.

Noncooperativity has been observed previously for bolaform amphiphiles[30,31] having a membrane-spanning spacer, probably due to the presence of gaps in the outer leaflets of the vesicles.[30,34,35] Due to this noncooperativity, it is difficult to define a precize phase transition temperature. We have, therefore, also determined the T_c of 12-24-12 with DSC, a method which does not depend on the use of a probe molecule. The DSC curves are shown in Figure 4.

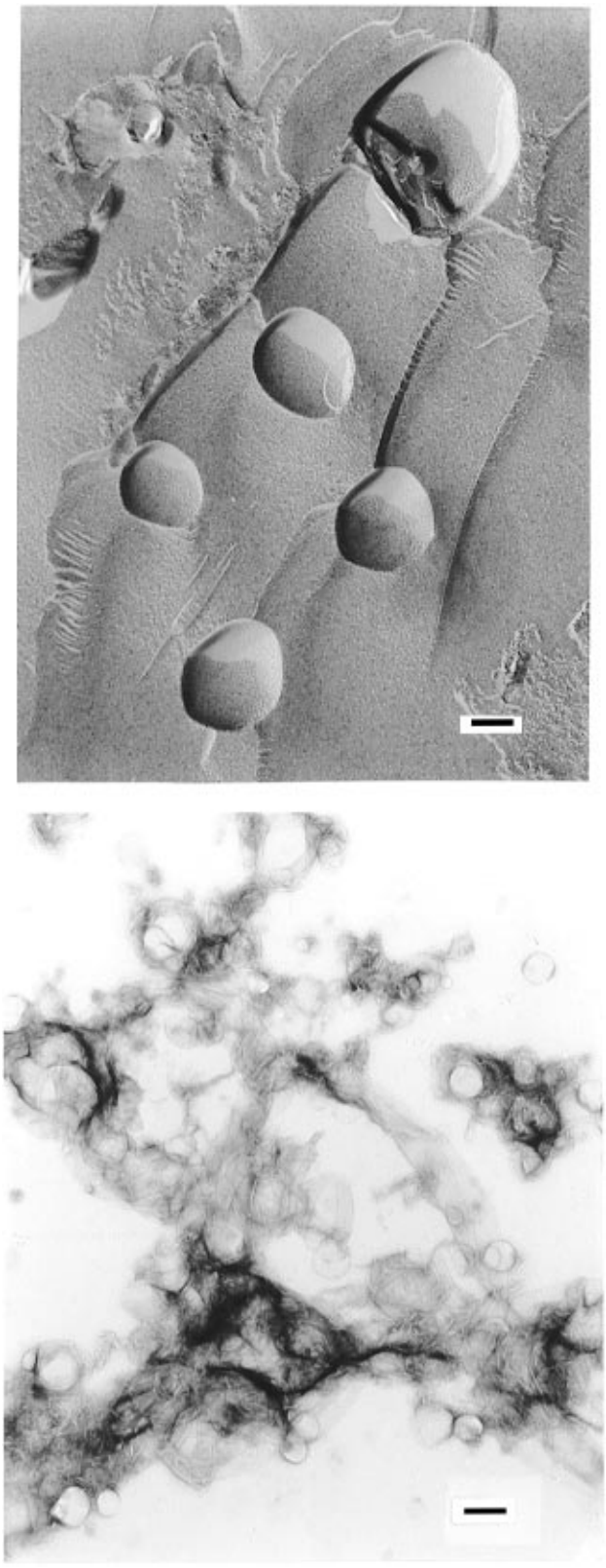

Figure 2. (a, top) Freeze fracture electron micrograph of vesicles of 12-24-12. Bar represents 100 nm. (b, bottom) Transmission electron micrograph of vesicles of 12-24-12. Bar represents 300 nm.

The result of the analysis of the curves[32,36] is listed in Table 2.

A fairly cooperative transition at 60.5 °C is observed, with a transition enthalpy of 32.7 ± 0.8 kJ (mol

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Table 1. Main Phase Transition Temperatures of the 12-*s*-12 Surfactants[a]

compound	fluorescence depolarization	^{1}H-NMR	^{31}P-NMR	DSC
12-6-12	*c*	*g*	*g*	*g*
12-8-12	*c*	*g*	*g*	*g*
12-12-12	47.5 (35−60)[b,d]	*g*	*g*	*g*
12-18-12	48.5 (22−75)[b]	*g*	*c*	*g*
12-24-12	45 (26−64)[b] 44 (30−58)[e]	59 (48−70)[f]	69 (63−75)[e] 67 (62−72)[f]	60.5[e]

[a] Listed values are the midpoints of the transition range, listed between brackets. [b] EtOH injection method in buffer. [c] No T_c measurable. [d] At 6.6×10^{-5} M. [e] Stirring method in double-distilled water. [f] Stirring method in D_2O. [g] Not determined.

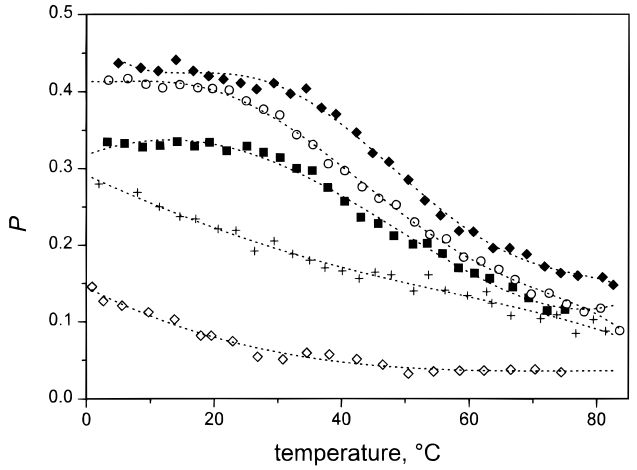

Figure 3. Results of the fluorescence depolarization experiments for 12-18-12 (○), 12-24-12 (■), 12-6-12 (+), and 12-12-12 (◆, 6.6×10^{-5} M; ◇, 7.8×10^{-4} M).

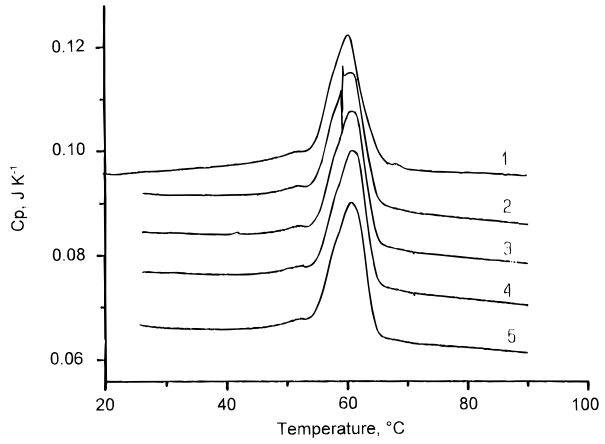

Figure 4. DSC scans for 12-24-12. Scans 1−4 are heating scans obtained immediately after cooling to 20 °C; scan 5 is a heating scan after standing at 20 °C for 11 h.

monomer)$^{-1}$ (average of two independent analyses). A similar apparent discrepancy between the DSC and fluorescence depolarization measurements has been found before.[32] Its origin can be attributed to the noncooperativity of the phase transition. Since the DSC experiments involved heating and fluorescence depolarization measurements involved cooling scans, the onset of the inclination in the fluorescence depolarization plot at high temperature should indicate the actual T_c. For 12-24-12, this is *ca.* 64 °C, which is in better agreement with the DSC experiments.

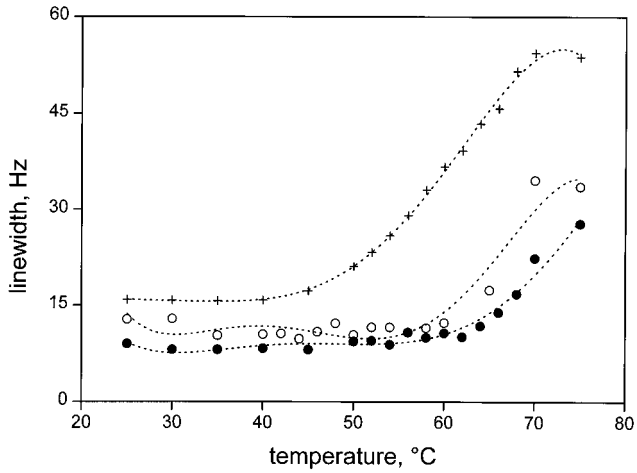

Figure 5. Results of the ^{1}H (+) and ^{31}P (○ in D_2O; ● in double-distilled water) NMR experiments for 12-24-12.

Fluorescence depolarization studies with the geminis gave rather capricious results. This is shown in Figure 3 as well.

For 12-6-12, a nearly straight line was obtained, characteristic for micelles. The polarization *P* at low temperatures, however, is rather high for micellar structures. The Krafft point lies below 0 °C, because no precipitation of 12-6-12 crystals at the lowest temperature was observed. Thus, the micelles of 12-6-12 appear to be less dynamic than conventional micelles. Similar results were obtained for 12-8-12 (not shown) and 12-12-12 at concentrations well above the cmc (*vide infra*). At concentrations slightly above the cmc, however, *P* gradually reached values > 0.4 at low temperatures, apparently indicating that the micelles become less dynamic at lower concentrations. This may be ascribed to probe-induced aggregation of the amphiphiles at low concentrations.

Attempts to determine the T_c of 12-24-12 with NMR line-broadening experiments were not successful. As is shown in Figure 5, line broadening occurs at high temperatures, which is opposite to the normal behavior.[33] This result is reproducible for both ^{1}H and ^{31}P NMR experiments. It looks as if the alkyl chains and the headgroups become more flexible below T_c, which, of course, conflicts with expectation.

Critical Aggregation Concentrations. The critical aggregation concentrations were determined using conductivity measurements, microcalorimetry, and spectroscopic methods (pyrene fluorescence[37] and pinacyanol chloride absorption[38]). The results are summarized in Table 3. It was not possible to determine critical vesicle concentrations (cvc's) for the bolaform amphiphiles 12-18-12 and 12-24-12 with either method, because they are probably too low for accurate experimental determination.

For the geminis, the conductivity plots show one clear break at the cmc. The obtained cmc's are 0.35, 0.48, and 0.06 mM for 12-6-12, 12-8-12, and 12-12-12, respectively. Figure 6 shows the conductivity plot for 12-6-12. The counterion binding constants β, estimated from the ratio of the slopes after and before the cmc, are small, 0.16 for 12-6-12, 0.38 for 12-8-12, and 0.12 for 12-12-12. Apparently, the headgroup repulsion within the micelle is small, probably due to the spacer, keeping the headgroups far apart from each other.

Spectroscopic determination of the cmc gave cmc values that are somewhat lower than those obtained from

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Table 2. DSC Measurements for 12-24-12[32,36]

					ΣH	ΣH_{int}	T_c	n
T in °C[a]	53	57.7	60.1	62.0				
H[a] in kJ (mol patch)$^{-1}$	372	950	1113	1142				
					3577	3573	60.8	109
T in °C[b]	53.4	57.9	60.5	62.1				
H[b] in kJ (mol patch)$^{-1}$	213	757	887	552				
					2409	2377	60.2	73

[a] Analysis of scans 2, 3, 4, and 5. [b] Analysis of the first scan.

Table 3. Cmc's (mM) of the 12-*s*-12 Surfactants Obtained by Different Methods[a]

s	conductivity	pyrene	pinacyanol chloride	microcalorimetry[d]	cmc ammonium[f]
6	0.35 ± 0.05	*b*	0.19 ± 0.03[c]	0.36 (0.17−0.53)	1.03[g]
	(β = 0.16)			ΔH_{mic} = −10.8[e]	1.12[h]
8	0.48 ± 0.04	0.15 ± 0.03[c]	0.10 ± 0.01[c]	0.17 (0.08−0.25)	0.83[g]
	(β = 0.38)			ΔH_{mic} = −11.7[e]	0.89[h]
12	0.06 ± 0.01	0.044 ± 0.003[c]	0.035 ± 0.002[c]	0.04 (0.02−0.05)	0.37[g]
	(β = 0.12)			ΔH_{mic} = −10.5[e]	0.28[h]

[a] Values for β are ±0.02; micellization enthalpies in kJ/mol. [b] Not determined. [c] Midpoint of the transition range. [d] Values between parentheses indicate the concentration range between the plateaus. [e] Micellization enthalpy at 30 °C in kJ/mol. [f] Cmc of the corresponding 12-*s*-12 bisammonium gemini surfactant. [g] Conductivity measurements.[6] [h] Surface tension measurements.[8]

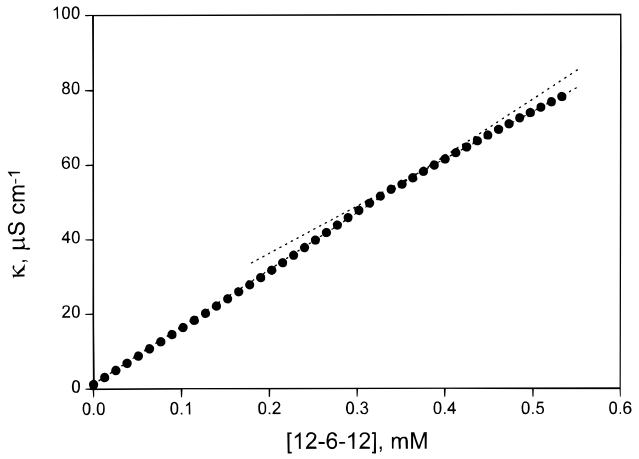

Figure 6. Typical plot of the conductivity *vs* surfactant concentration.

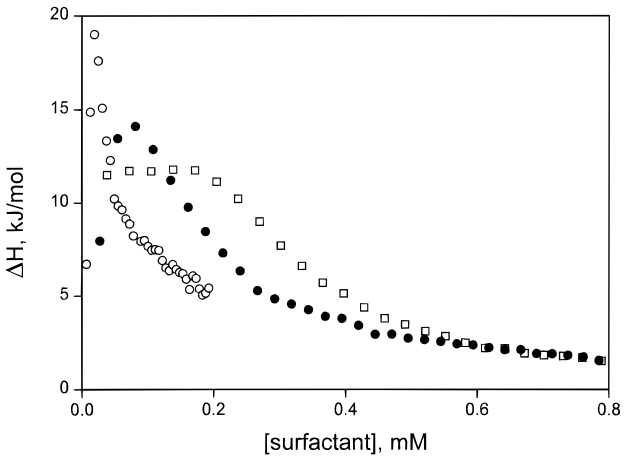

Figure 7. Result of the microcalorimetric study of the micellization of 12-6-12 (□), 12-8-12 (●) and 12-12-12 (○). Note the nonideality at the onset of the plots.

conductivity. This discrepancy may be attributed to probe-induced micellar aggregation, causing a local change in medium polarity[3,16] at concentrations just below the actual cmc. It has been argued before that conductometry indicates the end of the micellization process, whereas the spectroscopic methods indicate the onset of the micellization process,[16] because micellization occurs over a concentration range rather than at a fixed concentration.

The plots of the microcalorimetry experiments are shown in Figure 7. The cmc's, obtained from the enthalpograms, agree well with those obtained by the previous methods. The geminis display nonideal behavior below the cmc, which increases with the spacer length. It can be ascribed to the thermodynamic nonideality of the solutions,[39] giving rise to an endothermic peak.

The micellization enthalpies are nearly identical for all geminis. They are −10.8, −11.7, and −10.5 kJ/mol for 12-6-12, 12-8-12, and 12-12-12, respectively. The cmc's of the geminis are extremely low. This is characteristic for gemini-type surfactants. The cmc decreases upon increasing the spacer length. Zana *et al.*[6,8] have reported similar results for the corresponding ammonium geminis. The corresponding cmc's are listed in Table 3 as well for comparison.

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X-ray Powder Diffraction. In order to determine whether or not the spacer is membrane spanning in the vesicles, X-ray powder diffraction (XRD) studies were performed on dried amphiphile solutions. The reflections were interpreted as due to ordering of the material in stacked layers or membranes, with a *d*-spacing identical to the membrane thickness. From this value, a molecular picture of the packing of the membranes is proposed. The results are summarized in Table 4 and Figure 8.

12-18-12 showed a membrane thickness d = 40.9 Å, on the basis of the third-, fourth-, fifth-, and sixth-order reflections. Alternatively, by assigning the first observed reflection as being of second order, d = 27.2 Å, pointing to a membrane-spanning C_{18} spacer. Extension of our studies to the low-angle range unambiguously identified the first-order reflection corresponding to a *d*-spacing of 41.4 Å, which is consistent with a packing in bilayers of C_{12} chains. The 27.5 Å reflection, however, is very strong and probably indicates polymorphism with the presence of tilted C_{18} monolayers. It should also be noted that a very broad reflection was noted at low angle (d = 75.3 Å), which cannot be rationalized in terms of packing on the basis of the geometry of the molecule. Dehydration effects were noted when the relative humidity in the specimen chamber of the diffractometer was lowered. At 50% relative humidity, peaks corresponding to *d* values of 75.3, 41.4, and 27.5 Å were found. At 0% relative humidity, the 75.3 Å reflection was not affected, and at higher angles,

Table 4. XRD Results Related to the Geometry of the 12-*s*-12 Surfactants As Derived from CPK Models

s	geometry in Å[a]	*d* in Å[b]	alkyl tail cross section in Å^2	proposed packing[e]
8	25.5 (⊂), 23−25 (Z), 49 (ext)	23.9, 47.9	19.3	C_{12} mono- or bilayer
		34.7, 30.1, 24.0		intercalated bilayer
12	22−23 (Z), 55 (ext)	20.7	22.3	30° tilted C_{12} monolayer
18	20 (Σ), 31−32 (Z), 63 (ext)	40.9, 27.2	20.0	
		41.4,		hydrated C_{12} bilayer
		27.5[c]		hydrated 30° tilted monolayer
		40.8, 26.3[d]		C_{12} bilayer, tilted monolayer
24	37 (∩), 70 (ext)	38.6	19.7	
		38.8[c]		hydrated C_{24} monolayer
		36.3[d]		C_{24} monolayer

[a] ⊂, ∩, Σ, Z, see Figure 8a; ext = fully extended. [b] Underlined values have been obtained from low-angle measurements. [c] 50% relative humidity. [d] 0% relative humidity. [e] See Figure 8.

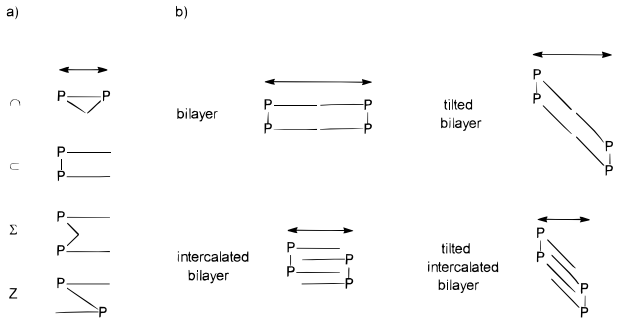

Figure 8. (a) Possible conformations of gemini/bolaform surfactants. (b) Possible modes of packing in bilayers. Arrows indicate the periodicity observed in an XRD experiment on stacked bilayers.

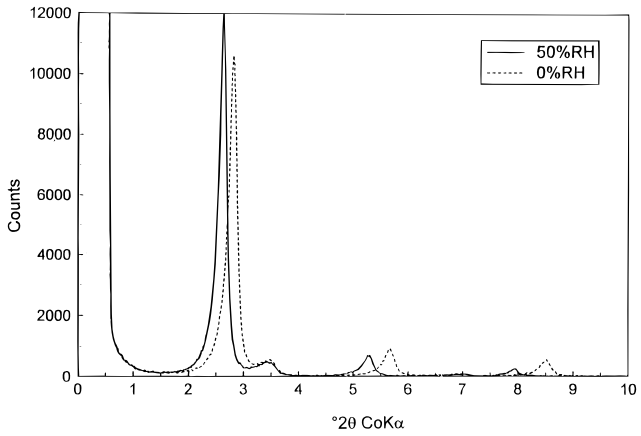

Figure 9. XRD (Co Kα radiation) of dried samples of 12-24-12 with controlled relative humidities (—, 50% RH; - - -, 0% RH).

additional 'shadow' peaks appeared at 40.8 and 26.3 Å, indicating slow dehydration.

For 12-24-12, the second-, third-, fourth-, and sixth-order reflections, corresponding to $d = 38.6$ Å, were found. This is consistent with a membrane-spanning spacer, as well as with a C_{12} bilayer. Some reflections were not accounted for by this interpretation. Extension of our studies to the low-angle range definitely established the presence of a first-order reflection of 38.8 Å. Variation of the relative humidity gave an indication of the origin of some reflections hitherto not accounted for. The diffraction patterns are shown in Figure 9. Diffraction peaks at 2θ values of 2.5−3°, 5−6°, and 7.5−9° are assigned to first-, second-, and third-order reflections, respectively, of periodicities of 38.8 Å (50% RH) and 36.3 Å (0% RH). Additional diffraction peaks at 2θ values of 3.5° and 7° are assigned to first- and second-order reflections of a less water-sensitive periodicity of approximately 30 Å, indicating some polymorphism in the sample. The difference in spacing points to the disappearance of a layer of one water molecule between the amphiphile layers.

The geminis 12-8-12 and 12-12-12 were also studied. A dried sample of 12-8-12 showed reflections, corresponding to *d* being either 23.9 or 47.9 Å, depending on their assignments. This points to a packing of non-intercalated monolayers or bilayers. Measurements on the low-angle diffractometer confirmed the *d* of 24.0 Å but did not give more positive evidence regarding the question whether this was a first- or second-order reflection, as no first-order reflection corresponding to a *d* of 48 Å was observed. The sample also showed polymorphism, with weak additional reflections at 34.7 (corresponding to an intercalated bilayer), 30.1, and 24.0 Å.

A dried sample of 12-12-12 showed a *d* of 20.7 Å, on the basis of the first-, second-, third-, and fourth-order reflections. This *d* is consistent with a packing in tilted C_{12} monolayers, with a membrane-spanning spacer. The presence of a tilt is corroborated by the slightly larger alkyl tail cross section, perpendicular to the bilayer normal, of 22.3 Å^2 (calculated from the d_s reflection at approximately 4.0−4.2 Å, using the relation[40,41] alkyl tail cross section $= 2d_s^2/\sqrt{3}$.

Conclusions

We have synthesized five new bolaform-type and gemini-type bisphosphate surfactants and studied their aggregation in aqueous solution. The geminis 12-6-12, 12-8-12, and 12-12-12 form micellar aggregates. The cmc's are very low, which is characteristic for geminis. The counterion binding constants β are low as well, indicating a small headgroup repulsion in the micelle. This has been attributed to the effect of the spacer, which keeps the headgroups far apart.

The bolaform amphiphiles 12-18-12 and 12-24-12 form vesicles in aqueous solution. Both surfactants display a noncooperative transition around 48.5 °C for 12-18-12 and 60.5 °C for 12-24-12. ^{1}H and ^{31}P NMR line-broadening studies gave unexpected results: line broadening occurred above the T_c.

XRD studies revealed that the spacer of 12-24-12 is indeed membrane spanning within the vesicular structure. For 12-18-12, the spacer is not membrane spanning.

It is clear that gemini and bolaform bisphosphate surfactants are an interesting class of amphiphiles with unusual properties. Further studies of their aggregation in aqueous solutions will be reported in due course.

Experimental Section

Materials. *n*-Dodecyl phosphate was prepared according to a standard literature procedure.[42] 1,6-Dibromohexane, 1,8-dibromooctane, 1,18-dibromooctadecane, and 1,12-dibromodode-

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cane were purchased from Janssen Chimica and used as received. Pyrene, pinacyanol chloride, NaAc, and HEPES were purchased from Aldrich and were used as received. The water used throughout the experiments was distilled twice in an all quartz distillation apparatus. NaAc/HEPES buffer (pH 7.4) was prepared by dissolving NaAc and HEPES in double-distilled water to give final concentrations of 5 mM NaAc and HEPES.

NMR spectra were recorded on a Varian Gemini 200 or a Varian VXR-300, using the chemical shifts of the solvents as internal standards. Infrared spectra were recorded on a Perkin-Elmer 841 infrared spectrophotometer as KBr pellets. Melting points were determined on a Mettler FP1 melting point apparatus equipped with an Ernst Leitz Wetzlar microscope 411657. Mass spectra were recorded using an AEI-MS9 mass spectrometer.

1,24-Dibromotetracosane. This compound was synthesized from 1,24-tetracosanedioic acid[43] by reduction with $LiAlH_4$ in THF under a N_2 atmosphere. The diol was converted into the dibromide in a HBr/AcOH mixture, according to a literature procedure.[44] ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 3.42 (t, J = 6.7 Hz, 4H); 1.83 (m, 4H); 1.27 (s, 40H). ^{13}C-NMR ($CDCl_3$, 200 MHz): δ 34.05, 32.81, 29.62, 29.42, 28.74, 28.15 (t).

12-*s*-12 Surfactants. These were synthesized according to the method described by Bauman.[25] In short, *n*-dodecyl phosphate (5 mmol) was dissolved in a 25% methanolic $N(CH_3)_4OH$ solution (containing exactly 10 mmol of $N(CH_3)_4OH$), and the methanol was removed *in vacuo* over a period of 20 min. The oily residue was taken up in acetonitrile (20−40 mL), and the α,ω-alkyl dibromide (2.5 mmol) was added with stirring. The reaction mixture was refluxed for 3−15 h (longer reflux periods for the longer alkyl dibromides) with the formation of a white precipitate. The solvent was then removed *in vacuo*, and the white solid was dissolved in water. The aqueous solution was acidified with HCl to a pH of < 0.5, and the white precipitate was collected on a Büchner funnel and washed thoroughly with water. The filtrate was extracted once with diethyl ether, and the bisphosphoric acid was crystallized from 96% ethanol, yielding the 12-*s*-12 surfactant in 80−90% yield. The bisphosphoric acids were subjected to the usual analytical methods. ^{1}H-NMR spectra were all as expected, with resonances at 4.05 ppm (q, CH_2O), 1.69 ppm (CH_2CH_2O), 1.27 ppm (s, $(CH_2)_n$), and 0.89 ppm (t, CH_3). In the ^{31}P-NMR spectra, a single resonance around 1.5 ppm was observed for all compounds. This chemical shift is concentration dependent. The IR spectra showed the characteristic absorption bands[25,26] around 1640 cm^{-1} (P−OH), 1215 cm^{-1} (P=O), and 1050 cm^{-1} (P−O−C). Elemental analyses were not satisfactory for the bisphosphoric acids 12-6-12 and 12-24-12, due to their hygroscopic properties.

12-6-12. Mp: 79.5−85.4 °C. Anal. Calcd for $C_{30}H_{64}O_8P_2$: C, 58.61; H, 10.49. Found: C, 57.23; H, 10.00.

12-8-12. Mp: 88.9−90.2 °C. Anal. Calcd for $C_{32}H_{68}O_8P_2$: C, 59.79; H, 10.66. Found: C, 59.19; H, 10.25. Exact mass: theoretical, 642.439; found, 642.439.

12-12-12. Mp: 88.9−91.2 °C. Anal. Calcd for $C_{36}H_{76}O_8P_2$: C, 61.86; H, 10.96; P, 8.86. Found: C, 61.50; H, 10.88; P, 8.74. Exact mass: theoretical, 698.502; found, 698.502.

12-18-12. Mp: 97.1−100.0 °C. Anal. Calcd for $C_{42}H_{88}O_8P_2$: C, 64.42; H, 11.33; P, 7.91. Found: C, 64.42; H, 10.99; P, 7.81. Exact mass: no M^+ peak observed.

12-24-12. Mp: 98.8−102.9 °C. Anal. Calcd for $C_{48}H_{100}O_8P_2$: C, 66.48; H, 11.62. Found: C, 67.59; H, 11.32. Exact mass: no M^+ peak observed.

All experiments were performed with the disodium salts of the bisphosphoric acids. These were prepared by dissolving the bisphosphoric acid in refluxing absolute ethanol with stirring and neutralizing the solution with an ethanolic NaOEt solution. The solution was refluxed for 15 min with stirring. When after this period the solution was not clear, the solution was filtered through a hard paper filter, using a preheated glass funnel. After removal of the ethanol *in vacuo* the white solid was crystallized from 96% ethanol. The salts were collected on a Büchner funnel, yielding the disodium salts in >95% yield. After the salts were dried *in vacuo*, they were ready for use. The purity was checked by IR spectroscopy.[26]

Vesicle Preparation. Vesicles were prepared at 60 °C, either in double-distilled water or in NaAc/HEPES buffers by means of two different methods: the ethanol injection method[27,28] or a stirring method. For the ethanol injection method, 5 mg of surfactant was dissolved in *ca.* 50 μL of 96% ethanol and 40 μL of this solution was injected rapidly with a preheated 100 μL Exmire syringe into 1 g of vigorously stirred double-distilled water or NaAc/HEPES buffer, which was placed in a water bath of 60 °C. Stirring was continued for a few minutes.

For the stirring method, *ca.* 5 mg of surfactant was added to 1 g of double-distilled water or NaAc/HEPES buffers and this suspension was stirred vigorously for a few minutes in a water bath at 60 °C. In some cases the solutions were placed in an ultrasonic water bath (at 60 °C) for 15−30 min afterward.

Electron Microscopy. For transmission electron microscopy (TEM), the two-droplet method was used for the preparation of the samples. A droplet of the surfactant solution was placed on a Formvar/carbon copper grid. After *ca.* 10 s, the grid was blotted off and a droplet of a 1% uranyl acetate solution was placed on the grid. This was blotted off again after *ca.* 10 s. The grids were air-dried before examination. For freeze fracture electron microscopy (FFEM), replicas were prepared using a Balzers EVM 052A electron beam evaporation instrument. Both TEM and FFEM samples were examined in a Philips EM 201 instrument, operating at 80 kV.

Fluorescence Depolarization. Into 5 mL of double-distilled water or NaAc/HEPES buffers, which was brought to the initial temperature of the experiment (>60 °C) was injected 50 μL of a surfactant solution were injected using a preheated 100 μL Exmire syringe. *trans,trans,trans*-1,6-Diphenyl-1,3,5-hexatriene (DPH) (5 μL of a 5×10^{-5} M solution in THF) was injected into the surfactant solution to yield a final [DPH] of 5×10^{-8} M. The experiments were started immediately after the preparation, using a quartz cuvet (1 cm). Samples of 12-6-12 and 12-8-12 were prepared by the stirring method in double-distilled water; samples of 12-12-12 and 12-18-12 were prepared by the ethanol injection method in NaAc/HEPES buffer; samples of 12-24-12 were prepared by both methods. Measurements were performed with an SLM-AMINCO SPF-500C spectrofluorometer. The emission and excitation wavelengths were 428 and 360 nm, respectively. A band-pass of 5 nm was used. The samples were thermostated by means of a water bath, and the samples were allowed to equilibrate for 10 min. Measurements involved cooling scans, with 3 °C intervals. *P* was calculated from the emitted light parallel and perpendicular to the direction of the excitation light, according to $P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$. Reported values for *P* are average values of five independent measurements.

NMR. The vesicle solutions were prepared at 60 °C by the stirring method in D_2O or in double-distilled water with 10% D_2O, followed by sonication for 15−30 min. Experiments involved cooling experiments. For the ^{1}H-NMR experiments, the line widths of the resonance peaks at 1.2−1.3 ppm were examined.

Differential Scanning Calorimetry (DSC). DSC measurements were performed at the University of Leicester with a Microcal calorimeter. A 4.35×10^{-3} M solution of 12-24-12 in water was heated to 60 °C with stirring for 15−20 min. After it was cooled to room temperature, the cloudy solution was degassed and scanned four times by immediately heating the solution after cooling to 20 °C. The fifth scan was performed after the solution was kept at 20 °C for 11 h. Analyses of the scans were carried out with the ORIGIN software.[32,36]

Conductivity. Inverse resistivities were measured using a Wayne-Kerr Autobalance Universal Bridge B642 fitted with a Philips electrode PW9512/01 (cell constant $C = 0.694$ cm^{-1}) or PW9512/00 (cell constant $C = 0.654$ cm^{-1}). Solutions were thermostated for at least 20 min prior to the experiment. Small aliquots (typically 10−50 μL) of a concentrated stock solution were injected into the cell with a 100 μL Exmire syringe, while stirring magnetically. Concentrations were corrected for volume changes. Conductivities κ were calculated from $\kappa = C/R = CG$.

Pyrene Fluorescence. A saturated solution of pyrene in water was prepared by adding *ca.* 2 mg of pyrene to *ca.* 50 mL of double-distilled water in a flask excluded from light. The mixture was vigorously stirred magnetically for 3 h at room temperature, and it was then allowed to stand overnight with gentle stirring.

A quartz cuvette was filled with the pyrene solution and

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equilibrated at 25 °C for at least 15 min. Small aliquots (typically 10−50 μL) of a concentrated stock solution were injected into the cell with a 100 μL Exmire syringe, while stirring magnetically.

Fluorescence measurements were performed with an SLM-AMINCO SPF-500C spectrofluorometer, with an excitation wavelength of 335 nm (band-pass 5 nm). Spectra were recorded in the ratio mode from 360−390 nm (band-pass 1 nm), by 0.1 nm steps. The first peak was taken at 372−373 nm, and the third one was taken at 383 nm. I_1/I_3 ratios were determined graphically.

Pinacyanol Chloride Absorption. A glass cuvette was filled with *ca.* 2.5 g of a 1.03×10^{-5} M pinacyanol chloride solution in double-distilled water. Into this solution, small volumes (typically 10−25 μL) were injected with a 100 μL Exmire syringe. The solution was stirred magnetically. Absorption was measured at 610 nm (bandwidth 1 nm). The temperature was controlled with a Haake water bath. Time intervals of 2 min were taken between the successive injections. Measurements were performed using a Philips PU 8740 UV/vis scanning spectrophotometer.

Titration Microcalorimetry. Microcalorimetric experiments were performed with an OMEGA Microcal titration microcalorimeter. Small aliquots (typically 5−10 μL) of a concentrated surfactant solution (*ca.* 10(cmc)) were injected into water. The heat effects were recorded and analyzed with a computer. Enthalpies were calculated using the ORIGIN software.

XRD Studies. Samples for the powder diffraction studies were prepared by dispersing the surfactant in water above the T_g (final concentration *ca.* 10 mg/mL) and putting a few drops of this suspension on a silica wafer, followed by lyophilization in a desiccator over P_2O_5. For the low-angle measurements, special Si single-crystal wafers, cut along the (501) plane, were used. Powder diffraction measurements were carried out on a commercial Philips PW 1710 X-ray powder diffractometer equipped with a Cu LFF X-ray tube operating at 40 kV and 55 mA. For low-angle measurements two Bragg−Brentano diffractometers were used,[45] *viz.* (a) an optimized home-built (NIOZ, Texel) high-accuracy $\theta-\theta$ diffractometer equipped with a Cu LFF tube, a variable divergence and antiscatter slit, and an energy dispersive Si/Li detector (Kevex), which enables a high peak to background ratio, and (b) a commercially available $\theta-\theta$ diffractometer (Philips), slightly adapted for measuring at low angles, equipped with a Co LFF tube, a variable divergence slit, a fixed antiscatter slit, a graphite monochromator in the diffracted beam, a detector of the Peltier-cooled Si/Li type, and a specimen chamber, the relative humidity (RH) of which can be controlled by a humidity generator providing for He gas of a desired RH value.[45]

Both instruments were operated at 40 kV and 40 mA.

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